spectroscopic consequences of slow rotational diffusion. Encapsulation of macromolecules within the protective nanoscale aqueous interior of reverse micelles dissolved in low viscosity fluids has been developed as a means through which the 'slow tumbling problem' can be overcome. This approach has been successfully applied to diverse proteins and nucleic acids ranging up to 100 kDa, considerably widening the range of biological macromolecules to which the comprehensive small protein solution NMR methodologies can be applied. Recently, we have expanded the library of surfactants used to encapsulate proteins and nucleic acids within the reverse micelle water core with high structural fidelity. In addition, new applications have emerged including studies of protein hydration, dynamic nuclear polarization in solution, structural characterization of integral membrane proteins and membrane-anchored lipidated proteins. The nanoscale interior of the reverse micelle water core also offers the potential for a range of novel applications in drug discovery. With the emergence of fragment based drug discovery, the screening of protein targets for binding to weak ligands has become the focus of some interest. NMR is well positioned to provide detailed site-specific information about ligand binding to proteins. The confined space of the reverse micelle allows a considerable reduction in ligand required for detection of weak binding and can also significantly extend the detection sensitivity into the low mM regime. Examples utilizing dihydrofolate reductase and aldoketoreductase will be presented. Supported by NIH grants R01 GM107829, T32 GM071339, T32 GM008275, F32 GM087099 and NSF grant MCB-115803.

3111-Pos Board B541

Sara: A Software Environment Supporting Rapid Acquisition and Analysis of NMR Relaxation Rates with Accordion Spectroscopy

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We present SARA (Software for Accordion Relaxation Analysis), an interactive and user-friendly MATLAB software environment designed for analyzing relaxation data obtained with accordion spectroscopy. Accordion spectroscopy can be used to measure nuclear magnetic resonance (NMR) relaxation rates more quickly than traditional methods, yet data analysis can be intimidating and no unified software packages are available to assist investigators. Hence, the technique has not achieved widespread use within the NMR community. SARA offers users a selection of analysis protocols spanning those presented in the literature thus far, with modifications permitting a more general application to crowded spectra such as those of proteins. We discuss the advantages and limitations of each fitting method and suggest a protocol combining the strengths of each procedure to achieve optimal results. In the end, SARA provides an environment for facile extraction of relaxation rates and should promote routine application of accordion relaxation spectroscopy.

3112-Pos Board B542

The Structure and Function of Supramolecular Self-Assembling Binary Guanosine Gels

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Binary liquid crystals of guanosine (GUO) and guanosine-5′-monophosphate (GMP) have been found to have unique and interesting properties in their ability to solubilize and selectively purify nanoparticles. The thermoassociative properties of these GMP-GUO g-gel phases have not been thoroughly explored and the mechanism of action is not understood. In the present study, nuclear magnetic resonance spectroscopy is used to probe the anisotropic properties of binary liquid crystals by employing deuterium nuclei. Deuterons have a quadrupolar moment that is normally averaged out by rapid molecular reorientation. In liquid crystals, however, the motion of the molecules is constrained such that quadrupolar effects are displayed in spectral data. To prevent other effects such as bulk exchange of deuterons from impacting the signal, $^{2}H_5$-labeled aniline is employed to study the supramolecular structure of the liquid crystalline gels. This isotopomer of aniline has deuterons that are strongly bound to a rigid aromatic ring, allowing for study of solvent directed interactions without concern for conformation or bulk exchange effects. This study determines the anisotropy and order parameter of the binary liquid crystals to elucidate the structural and functional properties of the system.

*This research is supported by the Photosynthetic Systems Program, Office of Basic Energy Sciences, United States Department of Energy (DE-FG02-07ER15903).

Electron Microscopy, Diffraction, and Scattering Techniques

3113-Pos Board B543

Cryo-Electron Tomography and Sub-Tomogram Averaging of Isolated Z-Discs from Honeybee Flight Muscle

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The Z-disc is the mechanical linkage that transmits the active and passive forces developed by muscle sarcomeres. It is also the location of proteins involved in diverse other processes, such as stress sensing into signaling pathways controlling muscle growth and wasting. About 40 different proteins are now known to be in the Z-disc, some to which are transient and relocate to other destinations such as the nucleus. The main route of force transmission is through pairs of antiparallel α-actinin Z-bridges; these link the overlapped ends of thin filaments from adjacent sarcomeres. The gross structure of the Z-disc varies widely between different muscles and species. Thickness varies in proportion to the number of registers of Z-bridges. The lattice of thin filament ends is hexagonal in invertebrates, whereas in vertebrates it is tetragonal. However the structure of the Z-disc is known only in outline to ~ 7nm resolution and the detailed layout of its components is mostly unknown. Methods to isolate Z-discs date back 50 years but such preparations have not been subjected to modern electron microscopy methods, such as cryo-EM, tomography or image processing. An advantage of isolated discs is they are thin, which obviates the need for sectioning for microscopy, which is damaging. We have prepared Z-discs from honeybee flight muscle using high salt extraction and density gradient purification. Vitrified preparations were examined in the 300 kV Krios microscope at the MRC Laboratory of Molecular Biology, Cambridge, UK. Tilt series images were recorded to +/− 70° with a Falcon II direct electron detector. 3D reconstruction and sub-tomogram averaging used Protomo software

3114-Pos Board B544

Label-Free Molecular Observations of Membrane-Associated Species using Backscattering Interferometry

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Membrane-associated proteins are integral components of cellular processes and disease pathogenesis. Quantitative observations of membrane protein interactions are extremely difficult; the membrane environment that is necessary to maintain appropriate structural and functional characteristics of such species interferes with or perturbs many analytical methods. In fact, typical assays to observe such interactions require the species of interest to be isolated and removed from the native membrane environment, usually with covalent modification. We will describe a label-free method to observe and evaluate membrane protein-ligand interactions in minimally-altered native membrane environments. This strategy is based on the use of backscattering interferometry (BSI) in which minute changes in the refractive index of the bulk solution caused by cognate ligand-receptor interactions are observed and quantified without the need for extrinsic molecular labeling. Combined with a method to present membrane proteins in an isotropically-scattering matrix derived from the native cellular environment, Ashlwe are able to observe a variety of cognate ligand-receptor interactions over a large range of equilibrium binding affinities.

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Stabilized, Non-Fouling Transmission Electron Microscopy Grid Coatings for the Selective Capture of His-Tag T7 Virus and His-Tag Gro EL from Cell Lysates

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Single-particle reconstruction has grown significantly with the improvements in various data collection and computational strategies including CTF fitting, the use of vitrified samples and the utilization of ultra-sensitive direct electron detectors. Although these improvements have contributed significantly to the recent evolution of 3D reconstruction analysis, the way samples are prepared for electron microscopy has remained largely unchanged. We report the development of TEM grids that are modified with a stabilized, non-fouling coating